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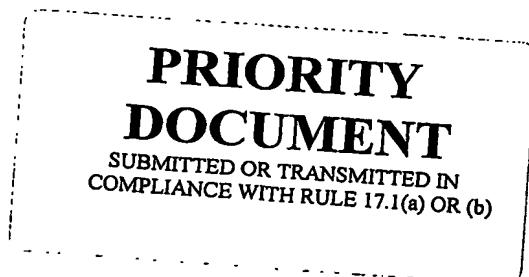
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I, LEANNE MYNOTT, MANAGER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2003900927 for a patent by BIODIEM LTD as filed on 28 February 2003.



WITNESS my hand this
Sixteenth day of March 2004

LEANNE MYNOTT
MANAGER EXAMINATION SUPPORT
AND SALES



AUSTRALIA
Patents Act 1990

PROVISIONAL SPECIFICATION

Applicant(s):

BIODIEM LTD

Invention Title:

GROWTH PROMOTION METHOD

The invention is described in the following statement:

GROWTH PROMOTION METHOD

FIELD OF THE INVENTION

This invention relates to a growth promotion method and in particular to the use of substituted nitrostyrene compounds in the promotion of growth of an animal, more specifically intensively farmed animals, such as, pigs, cows, sheep, chickens, turkeys or fish.

BACKGROUND

All references, including any patents or patent applications, cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. The discussion of the references states what their authors assert, and the applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country.

A popular method of livestock management is the intensive management system, which dramatically increases the population density of farmed animals and therefore improves the cost efficiency of housing, management and labour compared to less intensive management systems. However, a major consequence of intensive management is the increased opportunity for the development and spread of disease. This disease may be caused by specific pathogens introduced, for example, by errors in practice management, or by opportunistic pathogens in the contaminated and compromising intensively managed environment.

Clinical infections can lead to major disease outbreaks with high morbidity and/or mortality. Subclinical infections can interfere with the normal

metabolic process of an animal. For example, interference with the gastrointestinal function of an animal can interfere with its normal eating habits. This results in poor growth and feed efficiencies by the animal, which in turn results in less efficient animal production.

Most additives can, but do not always, improve feed use and rate of live weight gain by 3 to 8 percent. However, as the additives increase the cost of a diet, their inclusion will only be cost effective if the improved performance covers the cost of the additive and the same improvement could not have been obtained by a less expensive means, such as a change in management system or an improved strain of animal.

Current feed additives approved for use in livestock diets are generally administered at 1 to 175 mg/kg feed. Many feed additives must be withdrawn from the diet before slaughter in order to minimise the presence of residues of the additive in the meat of the animal when consumed.

Current additives used to promote growth of animals include the administration of antibiotics, vitamins and minerals including copper and arsenicals, and hormone regulation therapies. Classic examples include use of the arsenicals to control swine dysentery, coccidiostats to control coccidiosis in poultry, and vitamins which compensate for vitamin deficiencies. However, feeding these molecules to animals which will be consumed by humans often raises concerns on the effect to both humans and the environment as a result of the development of antibiotic resistant bacteria and the residues remaining in the animal and in the soil. For example, there has been evidence of copper residues in pig livers and also in soils treated with pig slurry.

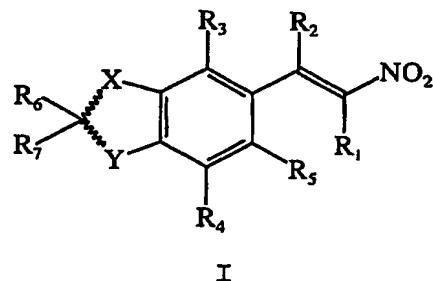
The development of antibiotic resistance in organisms is another major concern to consumers and compromises the ability of many widely used antibiotics to treat serious infections in humans. Not all antibiotics

promote growth. Therefore, the development of a growth promoting agent which leaves a minimal or no detectable residue is also of concern to consumers. Allergic reactions which occur as a result of consuming specific additives is known to occur. The effect of hormone supplements on consumers is of further concern and several hormone treatment programs in poultry and beef cattle have been restricted. There is increasing pressure from consumers to restrict the use of many compounds. In the European Economic Community, for example, non-authorised additives include tetracyclines, penicillins and cephalosporins, aminoglycosides, macrolides, sulfonamides and trimethoprim, nitrofurans except nitrovin, arsenicals, hormones and anti-hormones.

A requirement accordingly exists for growth promoting agents which leave minimal residues in animals to which they have been administered and in the environment. We have now found that certain substituted nitrostyrene compounds have improved growth promotant activity, require low dosage, result in low tissue levels, and have low oral toxicity.

SUMMARY OF INVENTION

In a first aspect, the present invention provides a method of promoting growth comprising the step of administering an effective amount of a compound of formula I:



in which

X and Y are either the same or different and selected from a heteroatom;

Y is a double or single bond depending on the heteroatoms X and Y ;

R_1 to R_5 are either the same or different and selected from hydrogen or a non-deleterious substituent, and

R_6 and R_7 are either the same or different and selected from hydrogen and a non-deleterious substituent or one of R_6 and R_7 are absent when there is a double bond present,

pharmaceutically or veterinarily acceptable salts or derivatives, pro-drugs, tautomers and/or isomers thereof to a subject in need thereof.

The present invention also provides the use of the compound of formula I in promoting growth of a subject.

The present invention further provides the use of the compound of formula I in the manufacture of a medicament for promoting growth of a subject.

The present invention still further provides the compound of formula I for use in promoting growth of a subject.

The promotion of growth may be determined by any suitable known method. In a preferred method, promotion of growth is determined by an increase in the weight, length, and/or height or a decrease in food intake and/or instance of microbial infection of the subject as compared to a control subject. In a more preferred method, promotion of growth is determined by an increase in the weight of an subject as compared to a control subject.

It will be understood that the compound can be administered by any means. Preferably, the compound is administered orally, topically, or parentally, more preferably orally.

Preferably X and Y are either the same or different and selected from O and N, more preferably both X and Y are oxygen.

Preferably R_1 and R_2 are either the same or

different and selected from hydrogen, hydroxy, halogen or optionally substituted C₁₋₆ alkyl.

R₃ to R₅ are preferably either the same or different and selected from hydrogen, hydroxy, halogen, nitro, C₁₋₆ alkoxy or optionally substituted C₁₋₆ alkyl.

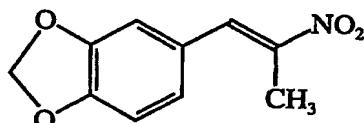
Preferably halogen is chlorine or bromine.

The E isomer of the compounds of formula I is preferred.

Particularly preferred are compounds of the formula I in which X, Y, R₆ and R₇ are as defined above; R₁ and R₂ are either the same or different and selected from hydrogen, hydroxy, Cl, Br and C₁₋₄ alkyl; and R₃ to R₅ are either the same or different and selected from hydrogen, hydroxy, Cl, Br, nitro, C₁₋₄ alkoxy or C₁₋₄ alkyl.

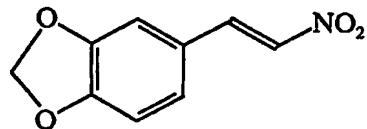
Specific examples of the compounds of the present invention are as follows:

(1) X and Y are O, R₁ is methyl and R₂ and R₃ are hydrogen (3,4-methylenedioxy- β -methyl- β -nitrostyrene) (hereinafter referred to as "Iksin")



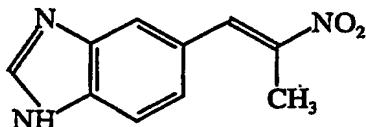
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(2) X and Y are O and R₁ to R₃ are hydrogen (3,4-methylenedioxy- β -nitrostyrene)

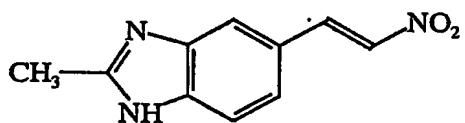


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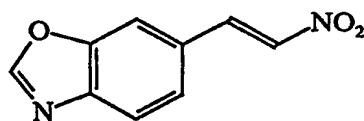
(3) X is N, Y is NH, R₁ is methyl and R₂ and R₃ are hydrogen (benzimidazole-5- β -nitropropylene)



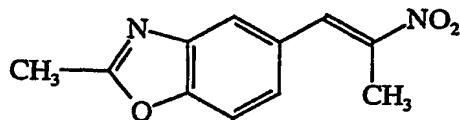
(4) X is N, Y is NH, R₁ is hydrogen, R₂ is methyl and R₃ is absent (2-methyl benzimidazole-5-β-nitroethylene)



(5) X is O, Y is N, R₁ and R₂ are hydrogen and R₃ is absent (benzoxazole-5-β-nitroethylene)



(6) X is N, Y is O, R₁ and R₂ are methyl and R₃ is absent (2-methyl benzoxazole-5-β-nitropropylene)



The subject may be of any type in which the promotion of growth is desired. As the compounds of formula I are broad-spectrum anti-microbial agents, they

are effective in inhibiting pathogens which effect a wide variety of subjects, thereby promoting growth of the subject.

The subject may be an animal or a human. In a preferred embodiment, the subject is an animal, more preferably an intensively farmed animal such as mammals, for example, pigs, cows or sheep; birds, for example, chickens, turkeys, ducks or geese; or fish.

In a second aspect, the present invention provides a composition for promoting growth in a subject, which comprises the compound of formula I and a carrier.

The composition may further comprise one or more other growth promoting agents. Other growth promoting agents include antimicrobials in reduced amounts, vitamins and/or minerals.

As the composition is generally to be administered to a human or animal it is preferably in the form of a pharmaceutical or veterinary composition comprising the compound of formula I and a pharmaceutically or veterinarianily acceptable carrier.

The pharmaceutically or veterinarianily acceptable carrier is preferably an organic solvent such as acetone, benzene, acetonitrile, DMSO or an alcohol, for example, methanol or ethanol. While the compounds of formula I show a poor solubility in water, when water is combined with an organic solvent a stable mixture is formed.

In a third aspect, the present invention provides a growth promoting agent comprising the compound of formula I.

The present invention also provides use of the compound of formula I as a growth promoting agent.

In a preferred embodiment of the invention, the compound of the formula I is administered to the subject in the form of a feed.

In a fourth aspect, the present invention provides a feed for promoting growth which comprises the compound of the formula I.

The compound of the formula I functions as a growth promoting agent and/or nutritional supplement in the feed.

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word "comprises" has a corresponding meaning.

The term "heteroatom" denotes O, N or S.

The term "non-deleterious substituent" is used herein in its broadest sense and refers to a substituent which does not have a deleterious effect on the growth promoting property of the compound. Examples include alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, aryloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloarylloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, diacylamino, acyloxy, alkylsulphonyloxy, arylsulphenyloxy, heterocyclyl, heterocycloxy, heterocyclamino, haloheterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy mercapto, alkylthio, arylthio, acylthio and phosphorus-containing compounds.

Particularly suitable non-deleterious substituents are alkyl, alkenyl, alkynyl, halo, haloalkyl, haloalkenyl, haloalkynyl, hydroxy, alkoxy, alkenyloxy, haloalkoxy, haloalkenyloxy, nitro, nitroalkyl, nitroalkenyl and nitroalkynyl.

In a preferred embodiment the non-deleterious substituents are C₁₋₆ alkyl, halo, hydroxy, C₁₋₆ alkoxy and nitro.

The term "halogen" refers to fluorine, chlorine,

bromine and iodine, preferably chlorine and bromine.

The term "alkoxy" is used herein in its broadest sense and refers to straight chain, branched chain or cyclic oxy-containing radicals each having alkyl portions, preferably C₁₋₆ alkyl, more preferably C₁₋₄ alkyl. Examples of such alkoxy groups are methoxy, ethoxy, propoxy, butoxy and t-butoxy.

The terms "C₁₋₄ alkyl" or "C₁₋₆ alkyl" used either alone or in compound words such as "optionally substituted C₁₋₄ or C₁₋₆ alkyl" refer to straight chain, branched chain or cyclic hydrocarbon groups having from 1 to 6 carbon atoms. Illustrative of such alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, neopentyl, hexyl, cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl.

The salts of the compound of formula I are preferably pharmaceutically acceptable, but it will be appreciated that non-pharmaceutically acceptable salts also fall within the scope of the present invention, since these are useful as intermediates in the preparation of pharmaceutically acceptable salts. Examples of pharmaceutically acceptable salts include salts of pharmaceutically acceptable cations such as sodium, potassium, lithium, calcium, magnesium, ammonium and alkylammonium; acid addition salts of pharmaceutically acceptable inorganic acids such as hydrochloric, orthophosphoric, sulphuric, phosphoric, nitric, carbonic, boric, sulfamic and hydrobromic acids; or salts of pharmaceutically acceptable organic acids such as acetic, propionic, butyric, tartaric, maleic, hydroxymaleic, fumaric, citric, lactic, mucic, gluconic, benzoic, succinic, oxalic, phenylacetic, methanesulphonic, trihalomethanesulphonic, toluenesulphonic, benzenesulphonic, salicylic, sulphuric, aspartic, glutamic, edetic, stearic, palmitic, oleic, lauric, pantothenic, tannic, ascorbic and valeric acids.

In addition, some of the compounds of the

present invention may form solvates with water or common organic solvents. Such solvates are encompassed within the scope of the invention.

By "pharmaceutically acceptable derivative" is meant any pharmaceutically acceptable salt, hydrate, ester or ether or any other compound which, upon administration to the subject, is capable of providing (directly or indirectly) a compound of formula I or a metabolite or residue thereof.

The term "pro-drug" is used herein in its broadest sense to include those compounds which are converted *in vivo* to compounds of formula I.

The term "tautomer" is used herein in its broadest sense to include compounds of formula I which are capable of existing in a state of equilibrium between two isomeric forms. Such compounds may differ in the bond connecting two atoms or groups and the position of these atoms or groups in the compound.

The term "isomer" is used herein in its broadest sense and includes structural, geometric and stereo isomers. As the compound of formula I may have one or more chiral centres, it is capable of existing in enantiomeric forms.

Without wishing to be bound by theory, it is believed that compounds of formula I act to promote the growth of a subject by inhibiting the growth of pathogens in the gastrointestinal tract as well as having a direct effect of feed utilisation.

It will be clearly understood that the terms "promotion of growth" and "promoting growth" mean the increase of growth of a subject when compared to a control subject.

As used herein, the term "control subject" means a subject of the same species, age and sex as the subject whose growth is being promoted however, the control subject has not been administered the compound of formula I.

The term "subject" as used herein refers to any subject having or at risk of having a disease or condition which requires growth promotion with the compound of formula I. The subject may be an animal or a human. While it is particularly contemplated that the compounds of the invention are suitable for treatment of domestic animals such as horses, ponies, donkeys, mules, llama, alpaca, pigs, cattle, sheep, birds and fish, or zoo animals such as primates, felids, canids, bovids, and ungulates, they are also applicable to use in humans and companion animals such as dogs and cats.

Preferably the animal is a mammal, bird or fish.

Suitable mammals include members of the orders Primates, Rodentia, Lagomorpha, Cetacea, Carnivora, Perissodactyla and Artiodactyla. Members of the orders Perissodactyla and Artiodactyla are particularly preferred because of their economic importance.

For example, Artiodactyla comprises approximately 150 living species distributed through nine families: pigs (Suidae), peccaries (Tayassuidae), hippopotamuses (Hippopotamidae), camels (Camelidae), chevrotains (Tragulidae), giraffes and okapi (Giraffidae), deer (Cervidae), pronghorn (Antilocapridae), and cattle, sheep, goats and antelope (Bovidae). Many of these animals, such as goats, sheep, cattle and pigs have very similar biology and share high degrees of genomic homology.

The order Perissodactyla comprises horses and donkeys, which are both economically important and closely related. Indeed, it is well known that horses and donkeys interbreed.

Preferably the mammal is a pig, cow or sheep.

Examples of birds include members of the order Anseriformes, which includes geese and ducks; Galliformes, which includes chickens, turkeys, quail, pheasants, guinea fowl and pea fowl; and Columbiformes, which includes pigeons and doves. Preferably the bird is a chicken,

turkey, duck or goose.

Examples of fish include Clupeiforms, Perciformes, Gadiformes, Pleuronectiformes, Cypriniformes, Crustaceans and Molluscs.

As used herein, the term "farmed intensively" means farmed with the aim of achieving maximum production within a limited area.

As used herein, the term "effective amount" is meant an amount of a compound of the present invention effective to yield the desired growth promoting activity.

The specific "effective amount" will, obviously, vary with such factors as the particular condition being treated, the physical condition of the subject, the type of subject being treated, the duration of the treatment, the nature of concurrent therapy (if any), the specific formulations employed and the structure of the compound or its derivatives.

The compounds of the present invention may additionally be combined with other molecules to provide an operative combination. It is intended to include any chemically compatible combination of pharmaceutically- or veterinarianily-active agents, as long as the combination does not eliminate the activity of the compound of formula I. It will be appreciated that the compound of the invention and the other molecule(s) may be administered separately, sequentially or simultaneously.

Other molecules which may be used to promote growth include other antimicrobials in reduced amounts, vitamins and minerals.

As used herein, a "pharmaceutical carrier" is a pharmaceutically acceptable solvent, suspending agent or vehicle for delivering the compound of formula I to a subject. The carrier may be liquid or solid and is selected with the planned manner of administration in mind. Each carrier must be pharmaceutically or veterinarianily "acceptable" in the sense of being compatible with other ingredients of the composition and non-

injurious to a subject.

The compound of formula I may be administered orally, topically, or parenterally in dosage unit formulations containing conventional non-toxic pharmaceutically or veterinarily acceptable carriers, adjuvants, and vehicles. The term parenteral as used herein includes subcutaneous injections, aerosol for administration to lungs or nasal cavity, intravenous, intramuscular, intrathecal, intracranial, injection or infusion techniques.

The present invention also provides suitable topical, oral and parenteral pharmaceutical or veterinary formulations for use in the methods of the present invention. The compounds of the present invention may be administered orally as a feed, or as tablets, aqueous or oily suspensions, lozenges, troches, powders, granules, emulsions, capsules, syrups or elixirs. As used herein, the word "feed" means food or drink.

The composition for oral use may contain one or more agents selected from the group of sweetening agents, flavouring agents, colouring agents and preserving agents in order to produce pharmaceutically or veterinarily elegant and palatable preparations. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharin. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, xanthan gum, bentonite, alginic acid or agar. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable preservatives include sodium benzoate, vitamin E, alphatocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate. The tablets contain the active ingredient in admixture with non-toxic pharmaceutically or veterinarily acceptable excipients

which are suitable for the manufacture of tablets.

These excipients may be, for example, (1) inert diluents, such as calcium carbonate, lactose, calcium phosphate or sodium phosphate; (2) granulating and disintegrating agents, such as corn starch or alginic acid; (3) binding agents, such as starch, gelatin or acacia; and (4) lubricating agents, such as magnesium stearate, stearic acid or talc. These tablets may be uncoated or coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. Coating may also be performed using techniques described in the U.S. Pat. Nos. 4,256,108; 4,160,452; and 4,265,874 to form osmotic therapeutic tablets for control release.

The compound of formula I as well as the pharmaceutically or veterinarianily active agent useful in the method of the invention can be administered, for *in vivo* application, parenterally by injection or by gradual perfusion over time independently or together. Administration may be intravenously, intraarterial, intraperitoneally, intramuscularly, subcutaneously, intracavity, transdermally or infusion by, for example, osmotic pump. For *in vitro* studies the agents may be added or dissolved in an appropriate biologically acceptable solvent or buffer and added to a cell or tissue.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media.

PARENTERAL VEHICLES include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, other antimicrobials, anti-oxidants, chelating agents, growth factors and inert gases and the like.

Generally, the terms "treating", "treatment" and the like are used herein to mean affecting a subject, tissue or cell to obtain a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or sign or symptom thereof, and/or may be therapeutic in terms of a partial or complete cure of a disease. "Treating" as used herein covers any treatment of, or prevention of disease in a subject, and includes: (a) preventing the disease from occurring in a subject that may be predisposed to the disease, but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; or (c) relieving or ameliorating the effects of the disease, i.e., cause regression of the effects of the disease.

The invention includes various pharmaceutical or veterinary compositions useful for ameliorating disease. The pharmaceutical or veterinary compositions according to one embodiment of the invention are prepared by bringing the compound of formula I, analogues, derivatives or salts thereof, or combinations of the compound of formula I and one or more pharmaceutically or veterinarily active agents into a form suitable for administration to a subject using carriers, excipients and additives or auxiliaries. Frequently used carriers or auxiliaries include magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, milk protein, gelatin, starch, vitamins, cellulose and its derivatives, animal and vegetable oils, polyethylene glycols and solvents, such as sterile water,

alcohols, glycerol and polyhydric alcohols. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobial agents, anti-oxidants, chelating agents and inert gases. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like, as described, for instance, in Remington's Pharmaceutical Sciences, 20th ed. Williams & Williams (2000), the British National Formulary, 43rd edition (British Medical Association and Royal Pharmaceutical Society of Great Britain, 2000), the contents of which are hereby incorporated by reference. The pH and exact concentration of the various components of the pharmaceutical or veterinary composition are adjusted according to routine skills in the art. See Goodman and Gilman's The Pharmacological Basis for Therapeutics (7th ed., 1985).

The pharmaceutical or veterinary compositions are preferably prepared and administered in dose units. Solid dose units may be tablets, capsules and suppositories. For treatment of a subject, depending on activity of the compound, manner of administration, nature and severity of the disorder, age and body weight of the animal, different daily doses can be used. Under certain circumstances, however, higher or lower daily doses may be appropriate. The administration of the daily dose can be carried out both by single administration in the form of an individual dose unit or else several smaller dose units and also by multiple administration of subdivided doses at specific intervals.

The pharmaceutical or veterinary compositions according to the invention may be administered locally or systemically in a therapeutically effective dose. Amounts effective for this use will, of course, depend on the severity of the disease and the weight and general state of the subject. Typically, dosages used *in vitro* may provide useful guidance in the amounts useful for *in situ*

administration of the pharmaceutical or veterinary composition, and animal models may be used to determine effective dosages for the promotion of growth. Various considerations are described, e.g., in Langer, *Science*, 249: 1527, (1990). Formulations for oral use may be in the form of hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin. They may also be in the form of soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.

Aqueous suspensions normally contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspension. Such excipients may be (1) suspending agent such as sodium carboxymethyl cellulose, methyl cellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; (2) dispersing or wetting agents which may be (a) naturally occurring phosphatide such as lecithin; (b) a condensation product of an alkylene oxide with a fatty acid, for example, polyoxyethylene stearate; (c) a condensation product of ethylene oxide with a long chain aliphatic alcohol, for example, heptadecaethylenoxycetanol; (d) a condensation product of ethylene oxide with a partial ester derived from a fatty acid and hexitol such as polyoxyethylene sorbitol monooleate, or (e) a condensation product of ethylene oxide with a partial ester derived from fatty acids and hexitol anhydrides, for example polyoxyethylene sorbitan monooleate.

The pharmaceutical or veterinary compositions may be in the form of a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to known methods using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may

also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The compounds of formula I may also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine, or phosphatidylcholines.

The compounds of formula I may preferably be presented for use in the form of veterinary compositions, which may be prepared, for example, by methods that are conventional in the art. Examples of such veterinary compositions include those adapted for: oral administration, external application, for example drenches (e.g. aqueous or non-aqueous solutions or suspensions); tablets or boluses; powders, granules or pellets for admixture with feed stuffs; pastes for application to the tongue; parenteral administration for example by subcutaneous, intramuscular or intravenous injection, e.g. as a sterile solution or suspension; or (when appropriate) by intramammary injection where a suspension or solution is introduced in the udder via the teat; topical applications, e.g. as a cream, ointment or spray applied to the skin; or intravaginally, e.g. as a pessary, cream or foam.

Dosage levels of the compound of formula I of the present invention may be of the order of up to about

1g per kilogram body weight. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage will vary depending upon the animal treated and the particular mode of administration. For example, a formulation intended for oral administration to humans may contain up to about 1 g of an active compound with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95 percent of the total composition. Dosage unit forms will generally contain between from about 5 mg to about 500 mg of active ingredient. When used in a feed, the amount of active ingredient will be about 10 ppm to about 100 ppm.

Optionally the compounds of the invention are administered in a divided dose schedule, such that there are at least two administrations in total in the schedule. Administrations are given preferably at least every two hours for up to four hours or longer; for example the compound may be administered every hour or every half hour. In one preferred embodiment, the divided-dose regimen comprises a second administration of the compound of the invention after an interval from the first administration sufficiently long that the level of active compound in the blood has decreased to approximately from 5-30% of the maximum plasma level reached after the first administration, so as to maintain an effective content of active agent in the blood. Optionally one or more subsequent administrations may be given at a corresponding interval from each preceding administration, preferably when the plasma level has decreased to approximately from 10-50% of the immediately-preceding maximum.

It will be understood, however, that the specific dose level for any particular subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and

the level of growth promotion required.

BRIEF DESCRIPTION OF THE DRAWINGS

In the example, reference will be made to the accompanying drawings in which:

Figure 1 is a graph showing plasma levels of Iksin in chicks after treatment with Iksin at 100 $\mu\text{g/g}$ in DMSO and at 400 $\mu\text{g/g}$ in distilled water/Tween20; and

Figure 2 is a graph showing the average percentage weight increase in chicks after single dosing with Iksin at 12 $\mu\text{g/g}$ in DMSO, 100 $\mu\text{g/g}$ in DMSO, 200 $\mu\text{g/g}$ in distilled water/Tween20 and 400 $\mu\text{g/g}$ in distilled water/tween20 compared to untreated chicks.

EXAMPLES

The invention will now be described in detail by way of reference only to the following non-limiting examples.

Example 1

Determination of dose range

Day-old chicks were orally administered Iksin in DMSO at 100 $\mu\text{g/g}$, 125 $\mu\text{g/g}$, 150 $\mu\text{g/g}$ and 200 $\mu\text{g/g}$ body weight and in Distilled Water (DW)/5% Tween-20 at 100 $\mu\text{g/g}$, 200 $\mu\text{g/g}$, 400 $\mu\text{g/g}$ and 500 $\mu\text{g/g}$ body weight.

The maximum tolerated oral dose of Iksin for day-old broiler chicks was 200 $\mu\text{g/g}$ Iksin/DMSO and ≥ 500 $\mu\text{g/g}$ in DW/T20.

Very little Iksin was absorbed from a single oral dosing. Iksin blood levels for doses of 12 $\mu\text{g/g}$ DMSO or 200 $\mu\text{g/g}$ DW were below the limits of detection. At 100 $\mu\text{g/g}$ DMSO and 400 $\mu\text{g/g}$ DW peak levels of 850 ng/g were observed at 48 h (Figure 1).

Effect on chick growth

Treatment with all doses and formulations of

Iksin resulted in increased weight gain in chicks 4 days after a single treatment (Figure 2). Chicks treated with Iksin 100 μ g/g DMSO showed a 32% increase in weight gain over the group of control chicks. Treatment with Iksin 12 μ g/g DMSO, 200 and 400 μ g/g DW/T20 gave a mean weight gain of 17%.

Conclusions

Oral administration of Iksin, formulated both in water and DMSO, is effective in promoting chick growth and is poorly absorbed with low tissue residues. It is anticipated that the results observed in the chicks will also be observed in other animals and humans.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

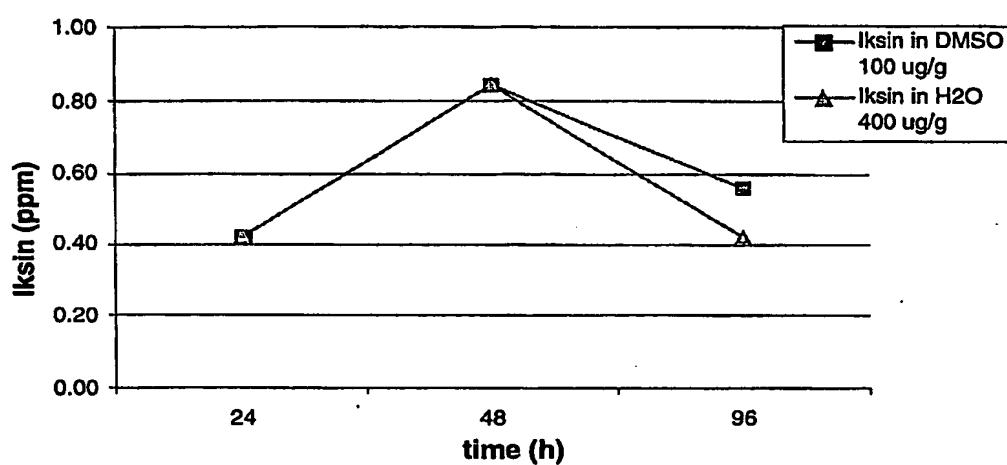


Figure 1

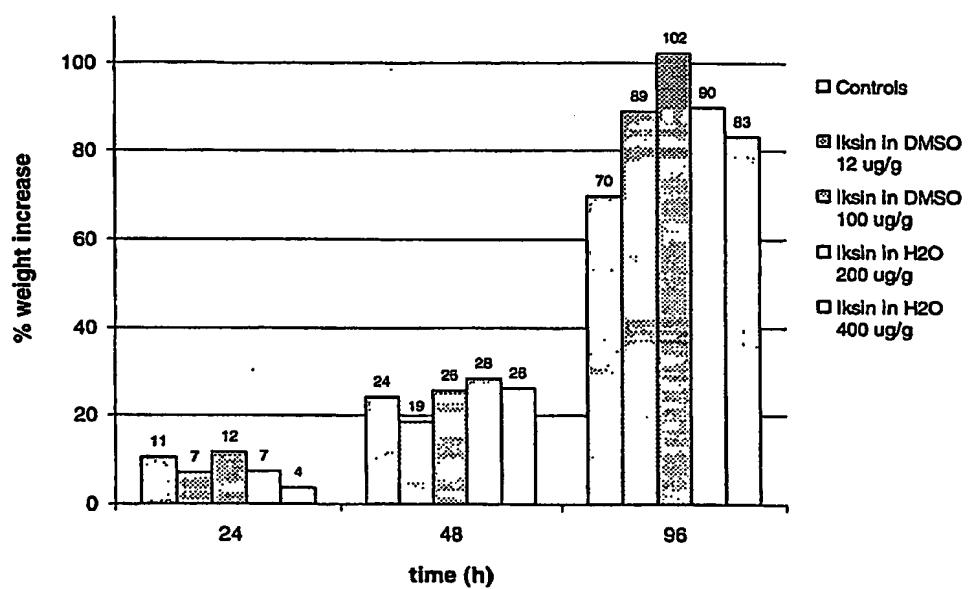


Figure 2